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TABLE 67  
In vitro Activity of Gemifloxacin against Gram-Negative  
Isolates from Surveillance Studies—All geographic Regions

Organism	Study	Number of Isolates	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)	MIC Range (µg/mL)
<i>Klebsiella pneumoniae</i>	GGSS	1820	0.03	0.5	0.002->256
	CAST	307	≤0.03	0.5	≤0.03->4
<i>Acinetobacter lwoffii</i>	GGSS	124	0.03	0.25	0.001-16
	CAST	6	≤0.03	NA	≤0.03-4.0

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TABLES 68 to 78 show the frequency distribution of gemifloxacin and comparator MICs for *P. vulgaris*, *K. pneumoniae*, *K. oxytoca*. The tables present the data combined from all three surveillance studies. Only the data from all geographic regions is shown.

Gemifloxacin has the lowest MICs tested against \_\_\_\_\_ with a MIC<sub>90</sub> of 0.03 µg/mL for the US/Canada isolates and 0.25 µg/mL for isolates from all geographic regions. These MIC<sub>90</sub> values are at least 2-fold lower than those of the other quinolones. Gemifloxacin's breakpoint, however, is at least 4 times lower than for the other quinolones. Ciprofloxacin and levofloxacin are the most active quinolones tested against \_\_\_\_\_. Gemifloxacin's MIC<sub>90</sub> is 4 times greater. Against *K. pneumoniae*, gemifloxacin's MIC<sub>90</sub> value is comparable to that of the other quinolones tested except for ofloxacin which has a higher MIC<sub>90</sub> value. Gemifloxacin's breakpoint, however, is 4 times lower. Gemifloxacin's MIC<sub>90</sub> value is two to eight times lower than that of the other tested quinolones against \_\_\_\_\_ and about the same as that of the other tested quinolones against \_\_\_\_\_. All quinolones demonstrated poor activity against \_\_\_\_\_ µS.

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TABLE 71  
Frequency Distribution of MICs for *Proteus vulgaris* in Surveillance Studies  
(all geographic regions)

N/Cum%	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	Total
Gemifloxacin	1 0.22	1 0.44	9 2.44	50 13.53	128 41.91	171 79.82	75 96.45	8 98.23	3 98.89	1 99.11	2 99.56	2 100*		451
Ciprofloxacin		4 0.89	157 35.70	223 85.14	40 94.01	7 95.57	8 97.34	3 98.00	7 99.56	3 100				451
Grepafloxacin			1 0.24	4 1.18	34 9.20	100 32.78	169 72.64	94 94.81	6 96.23	7 97.88	5 99.06	3 99.76	1 100	424
Levofloxacin			16 3.56	258 60.89	137 91.53	22 96.22	6 97.56	4 98.44	5 99.56	1 99.78	1 100			450
Ofloxacin					181 42.49	188 86.62	31 93.90	11 96.48	7 98.12	5 99.30		3 100*		426
Trovafoxacin			2 0.44	6 1.77	42 11.09	88 30.60	146 62.97	143 94.68	14 97.78	2 98.23	4 99.11	4 100*		451

\* Highest concentration tested in one or more of the studies.

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TABLE 72  
Frequency Distribution of MICs for *Klebsiella pneumoniae* in Surveillance Studies  
(all geographic regions)

N/Cum%	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	Total
Gemifloxacin	4 0.17	10 0.59	54 2.85	546 28.78	941 65.28	269 76.57	139 82.41	114 87.20	84 90.72	37 92.28	31 93.58	30 94.84	123 100*			2382
Ciprofloxacin			1 0.04	394 16.59	869 53.09	241 63.21	137 68.96	371 84.54	74 87.65	59 90.13	47 92.10	188 100*				2381
Grepafloxacin				153 8.41	745 49.34	429 72.91	110 78.96	86 83.68	69 87.47	46 90.00	24 91.32	23 92.58	14 93.35	42 95.66	79 100*	1820
Levofloxacin				32 1.34	613 27.08	1017 69.77	179 77.29	120 82.33	117 87.24	79 90.55	45 92.44	38 94.04	142 100*			2382
Ofloxacin						380 17.87	875 59.00	130 65.12	335 80.87	116 86.32	76 89.89	41 91.82	174 1008			2127
Trovafoxacin			2 0.08	74 3.19	635 29.86	924 68.67	211 77.53	107 82.02	117 86.94	79 90.26	48 92.27	37 93.83	147 100*			2381

\* Highest concentration tested in one or more of the studies.

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TABLE 73  
Frequency Distribution of MICs for *Klebsiella oxytoca* in Surveillance Studies  
(all geographic regions)

N/Cum%	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	Total
Gemifloxacin	1 0.12	4 0.58	60 7.51	295 41.62	341 81.04	71 89.25	33 93.06	23 95.72	5 96.30	9 97.34	11 98.61	3 98.96	9 100*			865
Ciprofloxacin			23 2.66	463 56.25	165 75.35	62 82.52	27 85.65	62 92.82	17 94.79	12 96.18	11 97.45	22 100*				864
Grepafloxacin				111 15.63	358 66.06	138 85.49	37 90.70	24 94.08	14 96.06	10 97.46	7 98.45	3 98.87	4 99.44	3 99.86	1 100*	710
Levofloxacin			1 0.12	36 4.28	452 56.53	234 83.58	53 89.71	23 92.37	23 95.03	15 96.76	9 97.80	7 98.61	12 100*			865
Ofloxacin						380 49.87	212 77.69	40 82.94	70 92.13	24 95.28	10 96.59	10 97.90	16 100*			762
Trovafloxacin			1 0.12	121 14.12	399 60.30	204 83.91	57 90.51	22 93.06	20 95.37	10 96.53	8 97.45	9 98.50	13 100*			864

\* Highest concentration tested in one or more of the studies.

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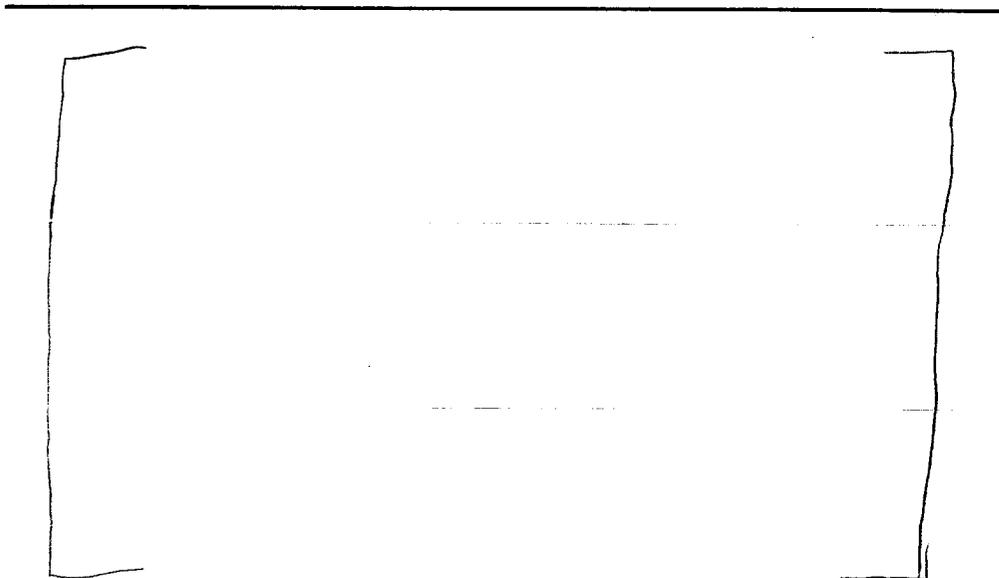
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Figures 14 to 24 are bar graphs that show the gemifloxacin data from the surveillance studies for \_\_\_\_\_ *P. vulgaris*, *K. pneumoniae*, *K. oxytoca*, \_\_\_\_\_ species. Only the data from all geographic regions combined is shown.

Figure 14—Frequency distribution of gemifloxacin MICs for \_\_\_\_\_  
From surveillance studies—all geographic regions (n = 4,163)



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Figure 17—Frequency distribution of gemifloxacin MICs for *P. vulgaris*  
From surveillance studies—all geographic regions (n = 451)

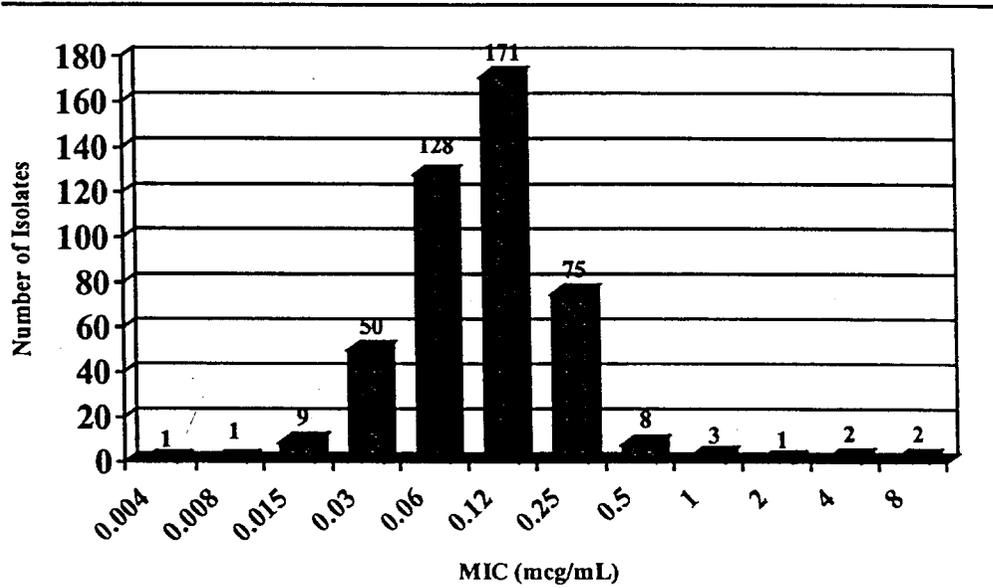
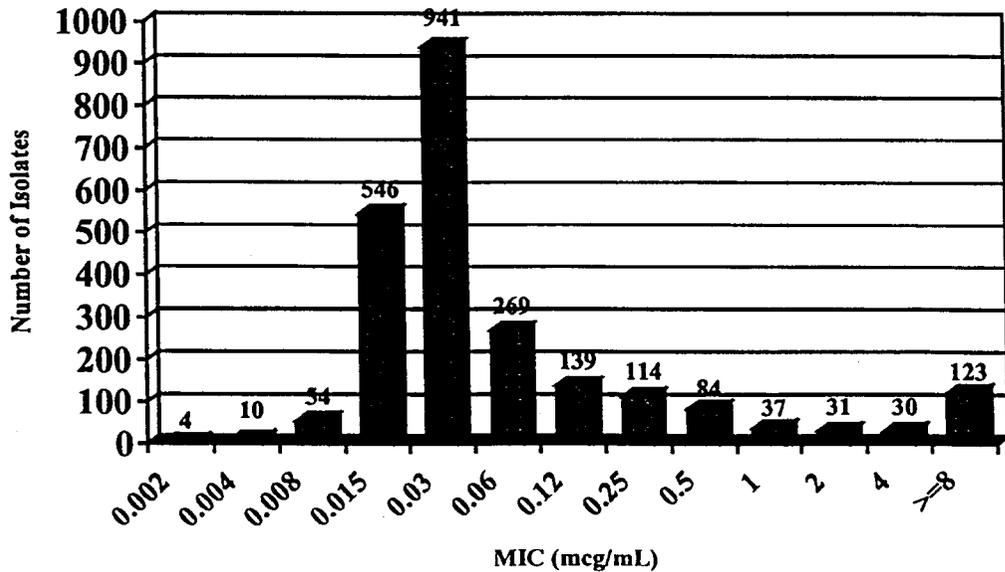


Figure 18—Frequency distribution of gemifloxacin MICs for *K. pneumoniae*  
From surveillance studies—all geographic regions (n = 2,382)



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Figure 19—Frequency distribution of gemifloxacin MICs for *K. oxytoca*  
From surveillance studies—all geographic regions (n = 865)

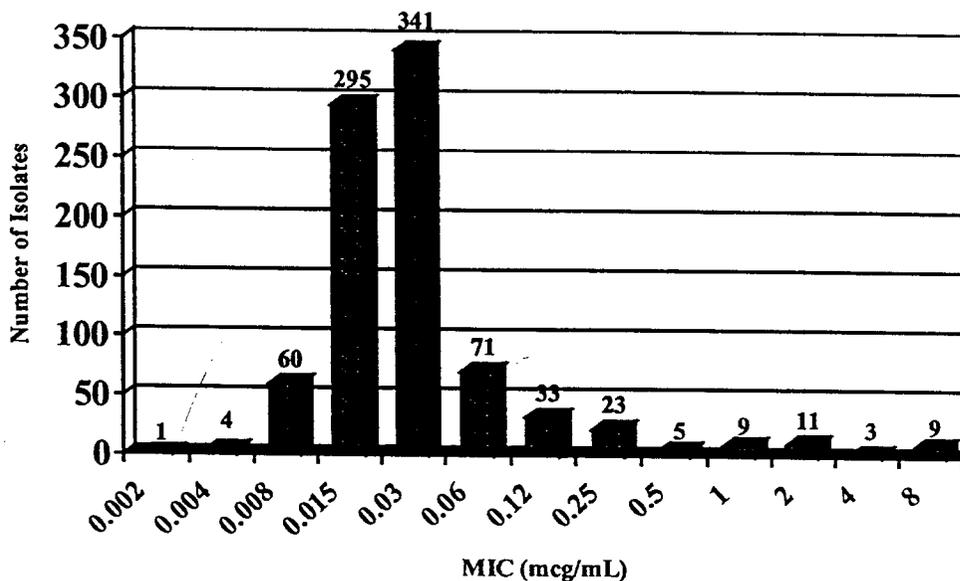
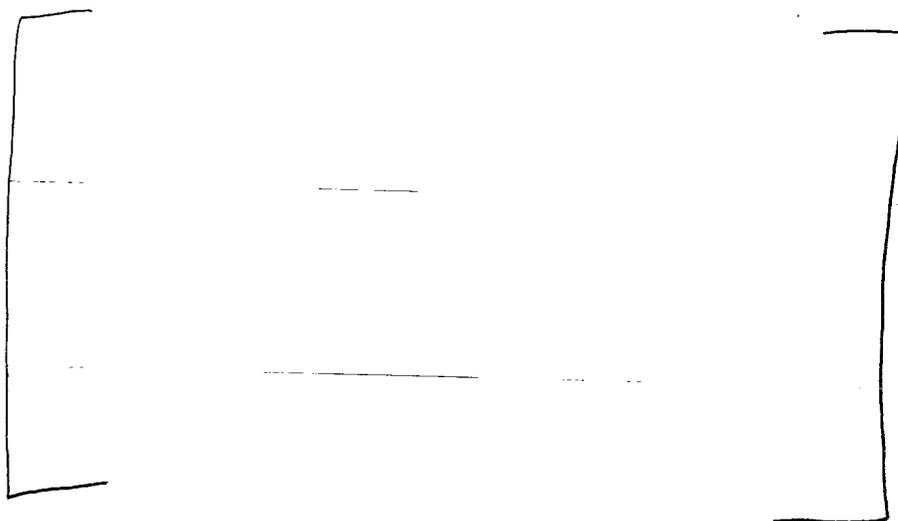


Figure 20—Frequency distribution of gemifloxacin for \_\_\_\_\_  
From surveillance studies—all geographic regions (n=1,141)



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Clinical data

This section contains frequency distributions of the gemifloxacin MICs for the key Gram-negative organisms isolated from the Intent to Treat population during the Phase III clinical studies. These data are presented in TABLES 79 to 84. The MIC<sub>90</sub> for \_\_\_\_\_ isolates recovered in North America and all geographic regions was 0.015 µg/mL. The MIC<sub>90</sub> for *K. pneumoniae* isolates recovered in North America and all geographic regions was 0.06 µg/mL. The MIC<sub>90</sub> for \_\_\_\_\_ isolates recovered in North America and all geographic regions was 4 µg/mL and 16 µg/mL, respectively.

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TABLE 81  
Frequency Distribution of MICs for Gemifloxacin for *K. pneumoniae*  
From the Phase III Clinical Studies (North America)

N=126	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Gemi n	2	22	69	17	8	3	1	1	1	0	0	1	0	1
Cum %	1.6	19.0	73.8	87.3	93.7	96.0	96.8	97.6	98.4	98.4	98.4	99.2	99.2	100

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TABLE 82  
Frequency Distribution of MICs for Gemifloxacin for *K. pneumoniae*  
From the Phase III Clinical Studies (all geographic regions)

N=171	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Gemi n	2	25	84	35	13	5	2	1	1	1	0	1	0	1
Cum %	1.2	15.8	64.9	85.4	93.0	95.9	97.1	97.7	98.2	98.8	98.8	99.4	99.4	100

TABLE 83

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Discussion

The studies outlined above demonstrate that gemifloxacin has variable *in vitro* activity against gram-negative organisms. Most species had MIC<sub>90</sub> values close to or at the susceptible breakpoint. The MIC<sub>50</sub> values in most studies were several dilutions lower than the MIC<sub>90</sub> values. The few studies that separated strains containing extended spectrum beta-lactamases (ESBLs) indicated that these strains were not susceptible to gemifloxacin. Certain species such as \_\_\_\_\_ species had MICs that were higher than for most other Enterobacteriaceae. In general the activity of gemifloxacin was equivalent to or one-dilution less than that of ciprofloxacin, but ciprofloxacin's susceptible breakpoint is 4 times higher.

*Acinetobacter lwoffii*—Over 100 isolates have been tested and more than one study has been performed. The MIC<sub>90</sub> values are below the susceptible breakpoint. *Acinetobacter lwoffii* may remain in the *in vitro* activity listing section of the label.

*Klebsiella pneumoniae*—Several studies have high MIC<sub>90</sub> values. Most of the other studies had MIC<sub>90</sub> values of 0.25 µg/mL (breakpoint concentration). This species is included in the clinical efficacy section of the label. If clinical efficacy is not shown then it must be eliminated from the label.

*Klebsiella oxytoca*—Over 100 isolates were tested. More than one study was performed. All MIC<sub>90</sub> values were ≤ 0.25 µg/mL. *Klebsiella oxytoca* may remain in the *in vitro* activity listing section of the label.

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*Proteus vulgaris*—Over 100 isolates have been tested. Only one small Korean study had a MIC<sub>90</sub> value greater than 0.25 µg/mL. *Proteus vulgaris* may remain in the *in vitro* listing section of the label.

### **ANAEROBIC MICROORGANISMS**

This section contains an overview of the *in vitro* activity of gemifloxacin against anaerobic organisms. The data is from only four studies.

In general gemifloxacin has some borderline activity against some Gram-positive species, such as *Peptostreptococcus*, some *Fusobacterium* species, and *Clostridium perfringens*. It has poor activity against most Gram-negative species such as *Bacteroides*. A summary of these results is shown in TABLE 85.

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TABLE 92  
Activity of Gemifloxacin Against

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ATYPICAL ORGANISMS

This section contains an overview of the *in vitro* activity of gemifloxacin against "so-called" atypical organisms. A primary proposed indication is respiratory tract infections, therefore, this section will focus on pathogens such as *Legionella pneumophila*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*. A summary of these results is shown in TABLE 93.

TABLE 93  
Summary of gemifloxacin and comparator activity against  
*Chlamydia*, *Mycoplasma*, and *Legionella* species from *in vitro* profile studies

Compound	No. of Isolates	MIC Range (µg/mL)	Range of MIC <sub>90</sub> s (µg/mL)	Median MIC <sub>90</sub> (µg/mL)	References
<i>Legionella pneumophila</i>					
Gemifloxacin	651	0.001-0.06	0.003-0.03	0.015	[69][67][66][70]
Trovafoxacin	651	0.0004-0.015	0.002-0.008	≤0.004	[69][67][66][70]
Levofloxacin	651	≤0.004-0.03	0.008-0.03	0.015	[69][67][66][70]
Ciprofloxacin	651	0.002-0.25	0.015-0.06	0.03	[69][67][66][70]
<i>Mycoplasma pneumoniae</i>					
Gemifloxacin	130	<0.008-0.12	0.12	NA <sup>1</sup>	[73]
Trovafoxacin	130	≤0.008-1	0.25	NA	[73]
Levofloxacin	130	0.03-8	0.5	NA	[73]
<i>Chlamydia pneumoniae</i>					
Gemifloxacin	25	0.004-0.25	0.25	NA	[72][71]
Trovafoxacin	25	0.06-1	1	NA	[72][71]
Levofloxacin	25	0.25-1	1	NA	[72][71]
Ciprofloxacin	5	0.25-0.5	NA	NA	[71]

<sup>1</sup> NA = Not applicable—insufficient data to determine a median value

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TABLES 94 to 96 show the results of individual studies for the species that the sponsor has included in the proposed labeling.

TABLE 94  
Activity of Gemifloxacin Against  
*Legionella pneumophila* (individual studies)

Compound	No. of Isolates	Country	MIC Range (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)	Reference
Gemifloxacin	7	USA	≤0.004-0.03	---	---	[65]
Gemifloxacin	100	UK	0.002-0.03	0.008	0.008	[66]
Gemifloxacin	204	Canada	0.008-0.06	0.016	0.016	[67]
Gemifloxacin	59	UK	0.004-0.015	0.008	0.008	[68]
Gemifloxacin	96	USA	≤0.002-0.015	0.004	0.008	[69]
Gemifloxacin	251	Spain	0.001-0.03	0.004	0.004	[70]

Over 100 isolates were tested. All studies had very low MIC<sub>90</sub> values. *Legionella pneumophila* may remain in the label. If clinical efficacy is proven it will be allowed in the clinical efficacy list. If not enough isolates are found in the clinical trials then it may be placed in list #2 (*in vitro* activity listing).

TABLE 95  
Activity of Gemifloxacin Against  
*Chlamydia pneumoniae* (individual studies)

Compound	No. of Isolates	Country	MIC Range (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)	Reference
Gemifloxacin	5	USA	0.004-0.008	---	---	[71]
Gemifloxacin	20	USA	0.125-0.25	0.25	0.25	[72]
Gemifloxacin	5	UK	0.06-0.12	0.12	0.12	[68]

Although only 30 isolates were tested, this is a fastidious organism and less than 100 isolates are sufficient to allow it into the label. All MICs were ≤0.25 µg/mL. This organism will be allowed in list #1 (clinical efficacy) if gemifloxacin is effective against it in the clinical trials. If not enough isolates are found in the clinical trials then it may be placed in list #2.

TABLE 96  
Activity of Gemifloxacin Against  
*Mycoplasma pneumoniae* (individual studies)

Compound	No. of Isolates	Country	MIC Range (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)	Reference
Gemifloxacin	130	USA	≤0.008-0.125	0.06	0.125	[73]
Gemifloxacin	85	UK	0.03-0.12	0.06	0.12	[74]

Over 100 isolates were tested. Two studies were performed. All MICs were ≤0.125 µg/mL. This organism will be allowed in list #1 if gemifloxacin is effective against it in the clinical trials. If not enough isolates are found in the trials then it may be placed in list #2.

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*Staphylococcus aureus*—Must be qualified with the statement “(methicillin-susceptible strains only).”

*Streptococcus pneumoniae*—If enough clinical evidence is shown then it may be listed in the first listing (clinical efficacy shown) as *Streptococcus pneumoniae* (including penicillin-resistant strains). The statement about macrolide-resistant strains must be deleted. If not enough penicillin-resistant strains (Pen MIC  $\geq 2$   $\mu\text{g/mL}$ ) are seen then this species will be listed in both the first and second listings. In list #1 it will be listed as *Streptococcus pneumoniae* (penicillin-susceptible strains only) and in list #2 as *Streptococcus pneumoniae* (penicillin-resistant strains).

*Haemophilus influenzae*—The statement “(including  $\beta$ -lactamase-producing)” must be deleted. Since quinolones are not beta-lactam type drugs this statement has not been allowed in any of the approved labels.

*Coxiella burnetti*—Will be allowed in list #1 (clinical efficacy) only. No *in vitro* data are included in the submission.

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The list of organisms should, therefore, read as follows:

Organisms with both clinical efficacy (if this is shown) and *in vitro* activity:

**Aerobic gram-positive microorganisms**

*Streptococcus pneumoniae* (penicillin-susceptible strains) may read (including penicillin-resistant strains) if clinical evidence is shown.

**Aerobic gram-negative microorganisms**

*Haemophilus influenzae*

*Haemophilus parainfluenzae*

*Klebsiella pneumoniae* (most strains are only moderately susceptible)

*Moraxella catarrhalis*

**Other microorganisms**

*Chlamydia pneumoniae*

clinical data only, no *in vitro* data available)

*Mycoplasma pneumoniae*

The *in vitro* activity list with MIC<sub>90</sub> values of  $\leq 0.25$   $\mu\text{g/mL}$  includes:

**Aerobic gram-positive microorganisms**

*Streptococcus pyogenes*

**Aerobic gram-negative microorganisms**

*Acinetobacter lwoffii*

*Klebsiella oxytoca*

*Proteus vulgaris*

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## EFFECT OF MISCELLANEOUS FACTORS ON ACTIVITY

Studies were performed to determine the effects of media and susceptibility testing method on the *in vitro* activity of gemifloxacin.

### EFFECT OF TEST MEDIUM

To evaluate the effects of media, the MICs of gemifloxacin, trovafloxacin and ciprofloxacin were determined using three different broth media: Brain Heart Infusion (BHI), Trypticase Soy (TSB) and the NCCLS recommended, cation adjusted Mueller Hinton (MH) (82). The drugs were tested against a panel of 90 clinical isolates. Results are shown in TABLE 97.

TABLE 97  
Comparison of MICs determined in different test Media

Drug	TBS	BHI
Gemifloxacin	94	97
Trovafloxacin	91	94
Ciprofloxacin	99	99

It appears that the type of media does not effect gemifloxacin MICs to any great extent.

### EFFECT OF TEST METHOD

To evaluate the effects of test methodology, \_\_\_\_\_  
\_\_\_\_\_ isolates. The results are shown in TABLE 98.

As is common with fluoroquinolones, \_\_\_\_\_  
\_\_\_\_\_ All three methods are, however, basically equivalent.

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### EFFECT OF CO<sub>2</sub> AND TEMPERATURE

The activity of gemifloxacin, trovafloxacin, and ciprofloxacin was determined against a panel of 100 clinical isolates (83). Each organism/drug combination was tested using standard NCCLS methodology at 37°C in normal atmospheric conditions, and in 5% and 10% carbon dioxide (CO<sub>2</sub>) and at 30° and 40°C. A trend towards an increase in MIC values was seen when gemifloxacin and ciprofloxacin were incubated in an environment containing 5% or 10% CO<sub>2</sub> as compared to normal atmospheric conditions, while a decreased MIC trend was observed with trovafloxacin. At an incubation temperature of 30°C there was a trend towards lower MIC values for gemifloxacin, trovafloxacin, and ciprofloxacin as compared to 37°C. At 40°C, the MICs of these compounds had a tendency to be higher. Overall, >90% of the gemifloxacin

These results are shown in TABLE 99.

TABLE 99  
Comparison of Various Incubation Conditions on MICs

Antimicrobial Agent	% MIC within ±1 dilution of standard method results			
	5% CO <sub>2</sub>	10% CO <sub>2</sub>	30°C	40°C
Gemifloxacin	92	91	91.7	88
Trovafloxacin	92	96	88.5	95
Ciprofloxacin	94	97	92.7	97

### EFFECT OF INOCULUM, PH, AND SERUM

Studies were performed to determine the effects of inoculum, pH, and serum on the *in vitro* activity of gemifloxacin. The MICs of gemifloxacin, trovafloxacin, and ciprofloxacin were determined against a panel of 100 clinical isolates (84). A high inoculum (10<sup>7</sup> CFU/mL) increased the MICs of all three drugs as much as 2 to >8-fold, while a low inoculum (10<sup>3</sup> CFU/mL) tended to decrease MICs 2 to 4-fold.

The addition of 50% human serum to the test medium tended to increase the MICs of gemifloxacin and trovafloxacin 2 to >8-fold. Trovafloxacin was affected much more than gemifloxacin. Even 25% human serum had some effect on trovafloxacin. A summary of this study is shown in TABLE 100. A trend toward higher MICs with increased inoculum and lower pH is often seen with quinolones.

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TABLE 100  
Comparison of inoculum, pH, and serum effects on Activity

Antimicrobial Agent	% MIC within $\pm 1$ dilution of standard method results					
	$10^3$ inoc.	$10^7$ inoc	pH 6.0	pH 8.0	25% serum	50% serum
Gemifloxacin	93	75	55	97	96	77
Trovafloracin	80	71	79	90	76	18
Ciprofloxacin	90	67	59	95	99	99

**EFFECT OF CATIONS**

A series of *in vitro* susceptibility studies were conducted to determine if the cationic strength of calcium ( $\text{Ca}^{++}$ ), magnesium ( $\text{Mg}^{++}$ ), or potassium ( $\text{K}^+$ ) in the test medium effect the antibacterial activity of gemifloxacin (85). The MICs of gemifloxacin were determined against a panel of 100 clinical isolates. Comparator compounds included ciprofloxacin and trovafloracin. MICs were determined and compared to results generated using the standard NCCLS recommended procedure for broth microdilution. At concentrations of 10 and 100 mM of  $\text{Mg}^{++}$ , as much as a  $\geq 8$ -fold increase in MICs was observed for gemifloxacin. No significant effects ( $\geq 95\%$  of all results within  $\pm 1$  dilution of the NCCLS results) on the antibacterial activity of gemifloxacin or the comparators were found with media containing 10 and 100 mM of  $\text{K}^+$  or 10 mM of  $\text{Ca}^{++}$ . This  $\text{Mg}^{++}$  effect (usually around concentrations of 9-10 mM) is seen with almost all fluoroquinolones. This effect at high  $\text{Mg}^{++}$  concentrations may be due to competition between  $\text{Mg}^{++}$  and the drugs for binding sites on the broken single-stranded DNA. Unsupplemented Mueller-Hinton medium is 0.3 mM in  $\text{Mg}^{++}$  concentration and normal human serum is about 1.1 mM. Changes in activity due to  $\text{Mg}^{++}$  only occur at much greater concentrations than those that would be seen clinically.  $\text{Ca}^{++}$  has been shown to also effect quinolone activity but usually only at concentrations of 50 mM or more. It is thus not surprising to see that 10 mM of  $\text{Ca}^{++}$  did not have any effect. The results are summarized in TABLE 101.

TABLE 101  
Comparison of cationic strength on Activity

Antimicrobial Agent	% MIC within $\pm 1$ dilution of standard method results				
	10 mM $\text{Mg}^{++}$	100mM $\text{Mg}^{++}$	10 mM $\text{Ca}^{++}$	10 mM $\text{K}^+$	100 mM $\text{K}^+$
Gemifloxacin	12.1	1	98	96	95
Trovafloracin	23.2	10.3	98	99	99
Ciprofloxacin	26.3	2.1	98	98	97

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Another study (86) tested the effect of human serum on the antibacterial activity of gemifloxacin against a panel of 22 anaerobic bacteria. The MICs of gemifloxacin, ciprofloxacin, ofloxacin, grepafloxacin, and trovafloxacin were determined using NCCLS procedures. In addition, MICs were determined in media supplemented with 25% and 50% human serum. In 25% serum, 95.5% of the gemifloxacin MICs were within  $\pm 1$  dilution of the MICs using standard testing. Similarly, >90% of the comparator quinolone results were within  $\pm 1$  dilution of those obtained using standard methods when tested in media containing 25% serum. The MIC values determined in media containing 50% serum were considerably more variable for all of the quinolones tested. At this higher serum concentration, 85.7% of the gemifloxacin, ciprofloxacin, and grepafloxacin results were within  $\pm 1$  doubling dilution. Scatter was greater for ofloxacin and trovafloxacin, with 76.2% and 71.4%, respectively within  $\pm 1$  serial dilution. Although the variability was greater at this higher serum concentration, trovafloxacin was the only compound significantly ( $p < 0.05$ ) less active.

**SUMMARY OF FACTORS THAT EFFECT *IN VITRO* ACTIVITY**

Gemifloxacin is similar to most other fluoroquinolones in that low pH has a significant effect on its MICs. High  $Mg^{++}$  concentrations also effects activity. High inoculum concentrations have some effect but not as much as low pH or high  $Mg^{++}$ .

**BACTERICIDAL ACTIVITY**

**RELATIONSHIP BETWEEN MIC AND MBC**

One method that is often used to see if a drug is bactericidal is to compare the MIC and MBC (minimal bactericidal concentration) against a series of organisms. In this method MBC is determined by subculturing broth from tubes without visible growth in the series of tubes used to determine the MIC. The MBC is defined as the concentration that produced a >99.9% ( $3 \log_{10}$ ) reduction in CFU. If the MIC and MBC are equal or within one doubling dilution the drug is usually considered bactericidal.

The MBCs of gemifloxacin were compared to those of ciprofloxacin and trovafloxacin against a panel of 139 clinical isolates (87). The MBCs of gemifloxacin, ciprofloxacin, and trovafloxacin were shown to be equivalent to or within a two-fold dilution of the MIC. The gemifloxacin  $MIC_{90}/MBC_{90}$  values ( $\mu g/mL$ ) were as follows: *S. aureus* (2/2), \_\_\_\_\_, *H. influenzae* (0.008/0.016), *M. catarrhalis* (0.008/0.008), \_\_\_\_\_, *K. pneumoniae* (1/1), and \_\_\_\_\_

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### KILL CURVES

Another method often used to determine the bactericidal activity of a drug is kill curves. In this method the drug is added to broth cultures of the bacterial strain at concentrations that are multiples of the MIC. Viable cells remaining after certain time periods such as 2, 4, 6, 8, and 24 hours are counted by inoculating agar plates with a portion of the growing culture. A 3 log<sub>10</sub> reduction in cell count is usually considered bactericidal.

Time kill studies were performed with gemifloxacin and ciprofloxacin against 8 isolates consisting of Gram-positive and Gram-negative species (87). Each isolate was incubated in the presence of ¼, ½, 1, 2, and 4 x MIC of gemifloxacin or ciprofloxacin. Viable counts (cfu/mL) were determined at 2-hour intervals for 8 hours and again at 24 hours. At 8 hours, gemifloxacin was bactericidal ( $\geq 3$  log reduction in cfu/mL) against all isolates at concentrations equivalent to or within a four-fold dilution of the MIC. At 24 hours, regrowth of the *S. aureus*, \_\_\_\_\_ isolates was observed. This is often seen with fluoroquinolones. Some cells are not killed and regrow after 24 hours. When tested these cells are not resistant. With the exception of \_\_\_\_\_ ciprofloxacin bactericidal effect was observed against the other isolates tested. Overall, these results indicate that gemifloxacin produces a bactericidal effect at concentrations equivalent to or within four-fold of the MIC.

In a similar study the bactericidal activity of gemifloxacin was determined against *S. aureus*, \_\_\_\_\_ and compared to ciprofloxacin (88). In this study gemifloxacin at the concentration of 1 or 2 x MIC, showed a rapid bactericidal effect against *S. aureus* 77. There was at least a 3-log<sub>10</sub> reduction in CFU/mL within 2 hours. The regrowth of *S. aureus* was prevented completely by gemifloxacin at 1, 2, and 4 x MIC. Regrowth occurred in the presence of 1 x MIC of ciprofloxacin. For \_\_\_\_\_ the bactericidal activity of gemifloxacin was also very potent. There was no detectable viable cells within 2 hours at 4 x MIC. \_\_\_\_\_ was killed rapidly by both gemifloxacin and ciprofloxacin. However, a persistent effect could only be demonstrated at four times the MIC.

In another study (40) the bactericidal activity of gemifloxacin and comparator drugs was investigated against 12 strains of *Streptococcus pneumoniae* (including two fluoroquinolone resistant isolates with ciprofloxacin MICs  $\geq 8$  µg/mL). Concentrations from ¼ to 8 x MIC for each drug were tested. All the fluoroquinolones behaved similarly, showing a concentration dependent rate of kill. Gemifloxacin and trovafloxacin were both bactericidal at their MIC concentration for the two fluoroquinolone-resistant strains (gemifloxacin MICs of 0.25 µg/mL and 0.5 µg/mL). Gemifloxacin was uniformly bactericidal ( $\geq 99\%$  kill) against all strains after 24 hours at 0.5 µg/mL. Although this is below the C<sub>max</sub> for gemifloxacin (1.2 µg/mL) this concentration will probably be considered to be above the susceptible range for gemifloxacin.

Appelbaum (49) studied the bactericidal activity of gemifloxacin and comparators against 10 isolates of *Haemophilus influenzae* at 1/2, 1, 2, and 4 x MIC. All fluoroquinolones behaved similarly, showing a concentration dependent rate of kill. All

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ten strains show at least a 1-log reduction in viable count at the gemifloxacin MIC by six hours, with 7/10 showing at least a 2-log reduction at the MIC. At 2 x MIC, gemifloxacin produced at least a 3-log reduction in viable count for all 10 isolates at 24 hours.

Time kill curves were used to compare the bactericidal activity of gemifloxacin to that of ciprofloxacin at various concentrations against *S. pneumoniae*, \_\_\_\_\_ and *H. influenzae* (89). The bactericidal activity of gemifloxacin was rapid and comparable to that of ciprofloxacin. Regrowth was prevented for all three strains for up to 24 hours. The MBC for gemifloxacin was 1 to 2 x the MIC. The results are shown in Figure 25.

The bactericidal activity of gemifloxacin was determined against \_\_\_\_\_ *S. aureus*, and *S. pneumoniae* (27). Gemifloxacin produced a characteristic bi-phasic dose-response curve in nutrient broth against all three bacterial species tested, which is typical of the fluoroquinolones. The optimum bactericidal concentrations for gemifloxacin against \_\_\_\_\_ *S. aureus*, and *S. pneumoniae* were 1.0, 0.5, and 0.5 µg/mL, respectively. Against *S. aureus*, gemifloxacin showed more potent bactericidal activity in nutrient broth than against \_\_\_\_\_. Against *S. pneumoniae*, the bactericidal activity of gemifloxacin in nutrient broth (supplemented with laked horse blood) was lower than that observed against either *S. aureus* or \_\_\_\_\_.

The above studies demonstrate that gemifloxacin is bactericidal. In most studies it is similar to most other fluoroquinolones.

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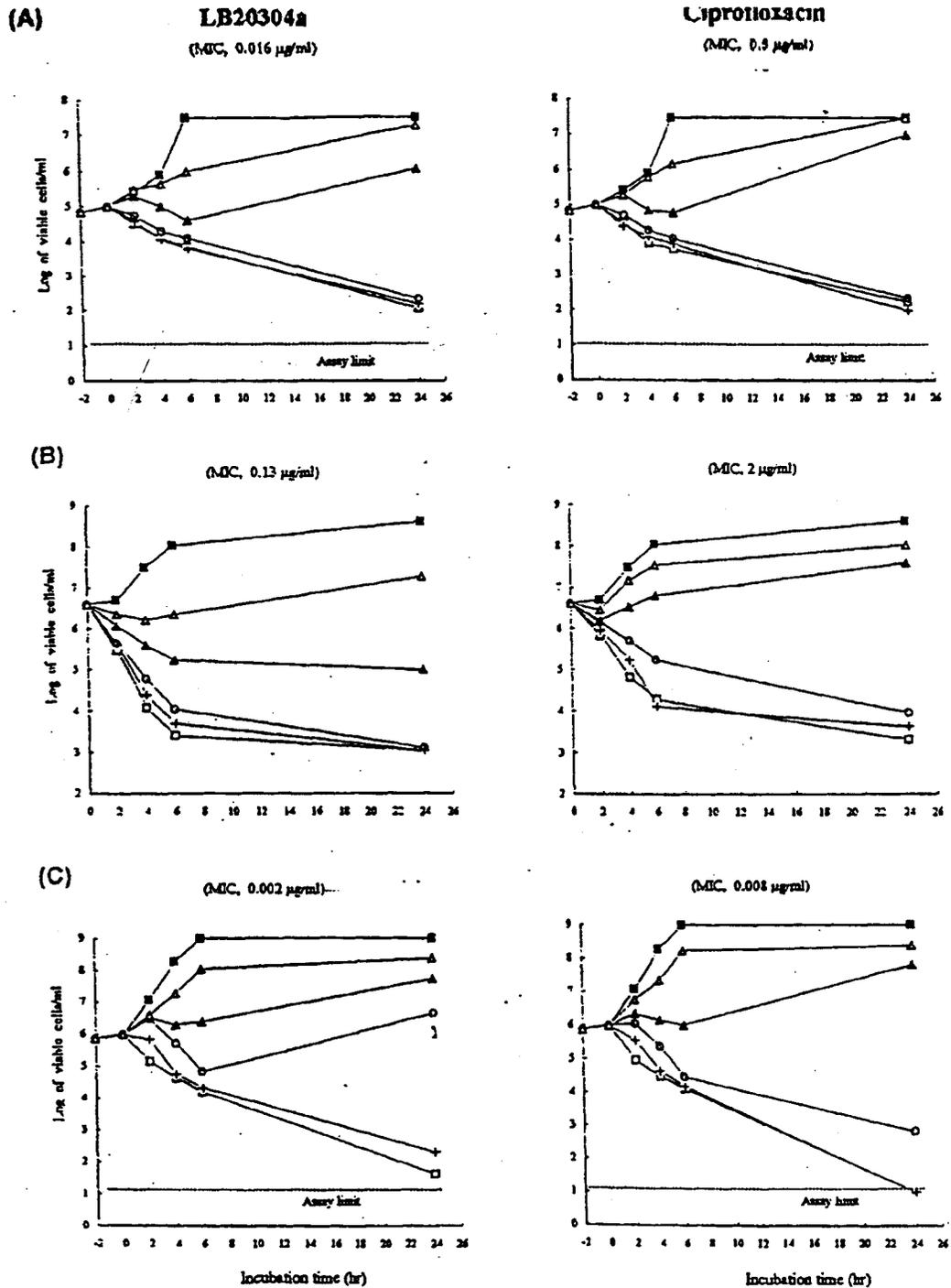
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Figure 25—Bactericidal Activity of Gemifloxacin and Ciprofloxacin  
Against *S. pneumoniae* (A), *S. pyogenes* (B) and *H. influenzae* (C)



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POST-ANTIBIOTIC EFFECT

The suppressive effect on bacterial growth that is seen following exposure to an antimicrobial agent and subsequent rapid removal of that agent is described as the postantibiotic effect (PAE).

$$PAE = T - C$$

Where T is the time required for a drug-exposed bacterial culture to increase 1 log<sub>10</sub> (10-fold) above the count observed immediately after drug removal and C is the time required for a drug-free control culture to increase 1 log<sub>10</sub> above the count observed immediately after completion of the same procedure used to remove drug from the test culture. In most of these studies rapid removal of the test drug is accomplished by drug inactivation, dilution of drug, filtration to remove drug but retain bacteria, or repeated washings of concentrated bacteria. Bacteria are normally quantitated by using a direct plate count.

The PAEs of gemifloxacin and ciprofloxacin were evaluated against *S. aureus* and \_\_\_\_\_ In this experiment centrifugation and resuspension removed the antibiotic. The PAE of gemifloxacin against *S. aureus* was comparable to that of ciprofloxacin with values at 1 x MIC and 2 µg/mL of 10 minutes and 93 minutes, respectively. Against \_\_\_\_\_ the PAE of gemifloxacin was similar to that of ciprofloxacin at 4 x MIC but was much longer than ciprofloxacin at a concentration of 2 µg/mL (19 hours for gemifloxacin versus 3.25 hours for ciprofloxacin).

In another study (91) the PAEs of gemifloxacin and ciprofloxacin were compared at 2 x and 4 x MIC against various species of bacteria. The gemifloxacin PAE at 4 x the MIC was >6 hours against *H. influenzae*, \_\_\_\_\_ and *P. vulgaris*, 0.5 to 1.5 hours against *S. pneumoniae*, *S. aureus*, *S. saprophyticus*, \_\_\_\_\_ and \_\_\_\_\_ It was only 12 minutes against *K. pneumoniae*. In most cases these values were comparable to those for ciprofloxacin or slightly shorter than those for ciprofloxacin.

Appelbaum (40) showed PAEs against *S. pneumoniae* of 0.4-1.6 hours (gemifloxacin), 0.5-3.7 hours (ciprofloxacin), 0.9-2.3 hours (levofloxacin), and 1.3-3.0 hours (trovafloxacin). In a further study (49), he reported that PAE against *H. influenzae* and \_\_\_\_\_ was similar or slightly lower than for other fluoroquinolones (0.3-2.3 hours). No PAEs were seen with the one fluoroquinolone-resistant isolate tested.

The intracellular PAE of a range of antimicrobials at 1 and 2 x MIC against *Legionella* species in human monocyte culture was studied (92). Gemifloxacin had one of the longest PAEs of all fluoroquinolones tested (3.5-4.6 hours) at 4 x MIC against *Legionella pneumophila*. The PAE for most other fluoroquinolones was 2 to 3.5 hours.

TABLE 102 summarizes the results of these PAE studies.

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TABLE 102  
Summary of Antibiotic PAEs following 1 hour Exposure to Drug

Organism	X MIC	Gemi	Cipro	Trova	Levo	Reference
<i>S. aureus</i>	2 x	1.1	1.3	---	---	[91]
<i>S. aureus</i>	4 x	1.0	1.5	---	---	[91]
<i>S. saprophyticus</i>	2 x	1.9	2.4	---	---	[91]
<i>S. saprophyticus</i>	4 x	2.5	3.9	---	---	[91]
<i>S. pneumoniae</i>	2 x	0.7	0.9	---	---	[91]
<i>S. pneumoniae</i>	4 x	1.5	1.5	---	---	[91]
<i>S. pneumoniae</i> (6)	10 x	0.5-1.6	0.5-1.5	1.3-3.0	0.9-2.3	[40]
<i>H. influenzae</i>	2 x	2.4	0.6	---	---	[91]
<i>H. influenzae</i>	4 x	> 6	2.4	---	---	[91]
<i>H. influenzae</i> (4)	10 x	0.3-2.3	1.3-4.2	0.8-2.8	2.8-6.2	[49]
<i>K. pneumoniae</i>	2 x	0.2	0.2	---	---	[91]
<i>P. vulgaris</i>	2 x	3.9	1.3	---	---	[91]
<i>L. pneumophila</i> (15)	4 x	3.49 ± 3	3.6 ± 2	1.71 ± 1	2.14 ± 2	[92]

Data on single strain except where n is specified

The above data demonstrate that gemifloxacin, like other fluoroquinolones has a postantibiotic effect. The duration of this effect is concentration dependent. The clinical significance of this effect is unknown, but a prolonged PAE may indicate that longer dosing intervals may still give satisfactory efficacy.

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## ANTIBACTERIAL INTERACTION WITH OTHER ANTIMICROBIALS

The combination of a given antimicrobial with another antimicrobial may effect the *in vitro* activity of either one resulting in a synergistic, indifferent, additive, or antagonistic effect. To determine the *in vitro* antibacterial effects of gemifloxacin in combination with other antimicrobial agents, a checkerboard assay was performed with gemifloxacin in combination with ten other antimicrobials (93). The drugs tested were amoxicillin, azithromycin, cefaclor, cefotaxime, chloramphenicol, gentamicin, meropenem, rifampicin, tetracycline, and vancomycin. The MIC values for individual compounds as well as the MIC of each compound in the presence of gemifloxacin were determined against a panel of ten bacterial isolates. The organisms tested were *S. pneumoniae*, *H. influenzae*, \_\_\_\_\_, *S. aureus*, \_\_\_\_\_, *K. pneumoniae*, *P. vulgaris*, \_\_\_\_\_. The combined effects were evaluated for synergistic/antagonistic activity by calculation of fractional inhibitory concentrations (FICs). This is calculated by the following equation:

$$\text{FIC} = \frac{\text{MIC of gemifloxacin in the combination}}{\text{MIC of gemifloxacin alone}} + \frac{\text{MIC of the other agent in combination}}{\text{MIC of the other agent alone}}$$

The sum of the FIC of both antimicrobials ( $\Sigma\text{FIC}$ ) expresses the extent of the interaction.  $\Sigma\text{FIC}$  values  $\leq 0.5$  indicates synergy;  $>0.5 \leq 1$ , an additive response;  $>1 \leq 2$ , indifference or no interaction; and  $>2$ , antagonism. Values between 0.5 and 2 are expected if little of no significant interaction is seen.

Most of the combinations resulted in additive or indifferent effects. Synergy was seen with combinations of cefaclor + gemifloxacin against \_\_\_\_\_ amoxicillin + gemifloxacin against \_\_\_\_\_ cefotaxime + gemifloxacin against \_\_\_\_\_ and meropenem + gemifloxacin against \_\_\_\_\_. None of the tested combinations produced an antagonistic effect.

The results of this combination study with gemifloxacin revealed results much like those seen with most other fluoroquinolones. Some strains and combinations yield synergistic results. Only one study was presented and in the case of other fluoroquinolones some studies often give results that differ for the same species drug combination. Most combinations in this study and studies with other fluoroquinolones show indifferent or additive results.

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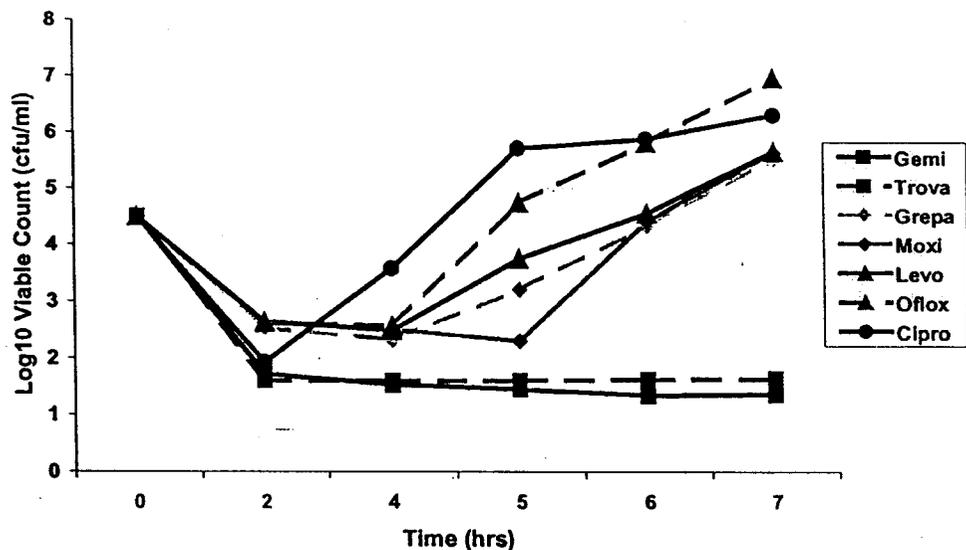
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### INTRACELLULAR ACTIVITY

With intracellular pathogens such as *Legionella* species, the antimicrobial agent has to be able to penetrate into the host cell before it can kill the infecting organism. Studies have been performed to determine the extent of penetration into the host cell and the ability of gemifloxacin to kill intracellular bacteria.

In one study (92) investigators studied the influence of antibiotics on two erythromycin-susceptible *Legionella pneumophila* strains, two erythromycin-resistant *L. pneumophila* strains, one strain of erythromycin-resistant *L. micdadei*, and one strain of erythromycin-resistant *L. longbeachae* multiplying intracellularly in mononuclear cell culture. Gemifloxacin, trovafloxacin, grepafloxacin, moxifloxacin, levofloxacin, ofloxacin, ciprofloxacin, azithromycin, clarithromycin, erythromycin, and rifampin were tested. Dosing was at 2 x MIC and viable counts were taken daily over 7 days. Gemifloxacin and trovafloxacin were the only antibiotics in this study with bactericidal activity against all strains (Figure 26) and this bactericidal activity continued even if the drug was removed from the medium after 1 day.

Figure 26—Intracellular Viable Counts for *L. pneumophila*



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In another study (65) it was shown that gemifloxacin accumulates rapidly in macrophages and can achieve intracellular levels up to 25-fold higher than extracellular concentrations after 60 seconds of incubation. After one hour of incubation about 40-50% of the drug egresses from the cells. Untreated macrophages supported a 2-log increase in the viable count of *L. pneumophila* over 24 hours, but pre-treatment with a

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fluoroquinolone at 0.25 µg/mL or 4 µg/mL generally resulted in a 6-7 fold reduction in viable cell count after 24 hours. The most pronounced reductions were seen with gemifloxacin and trovafloxacin. After 72 hours all the fluoroquinolones showed equivalent activity. Erythromycin at 0.25 µg/mL inhibited replication but did not result in a significant reduction in viable count over the 72 hour test period.

These data demonstrate that gemifloxacin, similar to other fluoroquinolones, has good activity against intracellular pathogens. Gemifloxacin has bactericidal activity against *Legionella pneumophila* while the bacteria are inside cells.

## ASSESSMENT OF RESISTANCE

### RESISTANCE MECHANISMS

#### Active Site Mechanisms

The primary mechanisms of bacterial resistance to fluoroquinolones can be attributed to mutations in the *gyrA* gene in gram-negative bacteria or the *grlA* (*parC*) gene in gram-positive bacteria. Mutations in the *gyrB* gene may also confer resistance, but to a lesser extent and less often than mutations in the *gyrA* gene. Another mechanism for decreased activity of quinolones is a reduction in the intracellular accumulation of drug by either a decrease in penetration of the drug or by an active membrane-associated efflux of drug from the cell.

The impact of mutations in the Quinolone Resistance Determining Region (QRDR) on gemifloxacin *in vitro* activity was investigated against *S. pneumoniae*, *S. aureus*, and \_\_\_\_\_

A collection of seventeen ofloxacin-resistant (MIC ≥8 µg/mL) clinical isolates of *S. pneumoniae* were characterized and the impact on gemifloxacin activity determined (94). A variety of substitutions were present in GyrA and both TOPO IV subunits (*parC* and *parE*) in 15 of the 17 strains investigated. In *gyrA*, 13 strains had mutations that resulted in a single amino acid substitution. In most cases this was S81F, but S81Y and E85K were also seen. A total of 15/17 strains had at least one amino acid substitution in *ParC* and 9 of these had two substitutions, the S79F + K137N pair was the most common. In *parE*, 13/17 strains had one substitution (I460V), with one strain showing two substitution. Only one strain had a mutation in *GyrB* (E474K). TABLE 103 summarizes the amino acid substitutions that were seen.

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TABLE 103  
Amino Acid Substitutions Found in Topoisomerases of Quinolone  
Resistant *S. pneumoniae* Strains

Organism	GyrA	GyrB	ParC		ParE	
Pt94 29123	S81F	----	S79F	----	I460V	----
403413	E85K	----	S79Y	K137N	I460V	----
402123	S81F	----	S79F	----	I460V	----
305313	----	----	S79Y	K137N	A532V	V535I
214152	S81Y	----	S79F	----	----	----
502226	S81F	----	D83N	----	I460V	----
509063	S81F	----	S79F	K137N	I460V	----
503244	S81F	----	S79Y	A189V	I460V	----
205229	S81F	----	S79F	K137N	I460V	----
203120	E85K	----	S79F	K137N	I460V	----
507103	S81F	----	R95C	----	D435N	----
622286	S81F	----	D83N	----	----	----
Pt94 1961	S81Y	----	S79F	K137N	I460V	----
503167	S81F	----	S79F	K137N	I460V	----
209165	----	----	----	----	----	----
L11259	----	----	----	----	I460V	----
205118	----	E474K	S79Y	K137N	I460V	----

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A statistic analysis was applied to the various fluoroquinolone resistance mechanisms. It is difficult to elucidate the effects of each individual target substitution, since the effects are often additive. It appears, however, that the most predictive substitution resulting in significant increases in MIC are those found around amino acid residues at positions 79 to 83 of ParC. Based on this statistical analysis, the rank order of ParC substitutions as they pertain to raising MICs is S79F=S79Y=D83N>R95C=WT. Efflux activity was apparent in varying degrees in all 17 isolates investigated. Efflux alone appeared to be the primary cause of resistance in only 3/17 isolates (L11259, 209165, and 507103). Of these, 209165 had no detectable target substitutions, L11259 showed one substitution in ParE (I460V), and 507103 showed substitutions in GyrA, ParC, and ParE (S81F, R95C, and D435N, respectively). In all three cases, resistance seems to be entirely attributable to efflux, since MICs of ciprofloxacin and norfloxacin in the presence of reserpine (an efflux pump inhibitor) are returned to baseline levels. Gemifloxacin MICs for these three strains were also effected by the presence of reserpine, indicating that gemifloxacin is a substrate for efflux pumps. Although *ParC* appears to be the primary target for gemifloxacin as is the case for most other fluoroquinolones, almost all strains also had a mutation in the *gyrA* gene. This may indicate that gemifloxacin has a strong affinity for both enzymes. It may, therefore, require mutations in both genes for high level resistance to develop against gemifloxacin.

Strains from a collection of penicillin-resistant *S. pneumoniae* from Northern Ireland with increased MICs to ciprofloxacin were characterized and the *in vitro* activity

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of gemifloxacin was determined (95). Two strains with ciprofloxacin MICs of 16 µg/mL harbored multiple topoisomerase mutations. The ParC S79F change was present in both strains. This substitution was associated with ParE D435V and GyrB E474K. Ciprofloxacin usually selects ParC changes rather than GyrA mutations. Gemifloxacin was found to have a MIC of 0.12 µg/mL against both of these ciprofloxacin resistant *S. pneumoniae* strains. The authors postulated that the relatively low impact of these multiple mutations on gemifloxacin activity (compared to ciprofloxacin MICs) was due to gemifloxacin primarily targeting GyrA rather than Topo IV (ParC). A study by Morrissey and George (23), however, suggest that Topo IV is the primary target. In their experiment the IC<sub>50</sub> values for all the tested quinolones was clearly less for topoisomerase IV than for DNA gyrase. As suggested in the previous discussion, these conflicting results may be due to the fact that gemifloxacin targets both sites almost equally.

A selection of ofloxacin-resistant (MIC ≥ 8 µg/mL) *S. pneumoniae* strains from the Alexander Project and a Northern Ireland strain collection were investigated in order to determine the factors contributing to fluoroquinolone resistance (96). Analysis of the QRDR regions of the 20 ofloxacin-resistant strains revealed a variety of mutations were present. The most frequent GyrA substitution was S81F. Five strains harbored a variety of substitutions in GyrB. ParC contained the most substitutions, K137N being the most frequent, encountered either alone or in combination with S79F or S79Y. The predominant ParE substitution was I460V. Substitutions I460V and K137N in ParC were also present in a large number of fluoroquinolone susceptible strains and thus appear to play a lesser role in high-level fluoroquinolone resistance. Substitutions S79F in ParC and S81F in GyrA appear to be associated with the highest average MIC. Gemifloxacin retained borderline *in vitro* (MICs of ≤ 0.12 µg/mL) activity against strains resistant to ofloxacin, ciprofloxacin, and levofloxacin.

A further study investigated the QRDRs of a representative number of strains from a collection of 57 levofloxacin-resistant *S. pneumoniae* strains (32). Strains possessing mutations in both *gyrA* and *parC* genes demonstrated high MICs for all fluoroquinolones tested, while MICs were usually only elevated two- to eight-fold by mutations in *parC* alone, or *parE* and *gyrA* combined. Unlike gatifloxacin and trovafloxacin, susceptibilities to gemifloxacin and clinafloxacin were less affected by mutations associated with resistance to levofloxacin.

In a Canadian study, the prevalence of pneumococci with reduced susceptibility to fluoroquinolones across Canada was determined (38). Isolates with ciprofloxacin MICs of 4 µg/mL were examined. The overall prevalence of pneumococci with reduced susceptibility to fluoroquinolones (PRSF) was 1.0%, which increased from 0% in 1993 to 2.1% in 1998. Reduced susceptibility to fluoroquinolones was associated with resistance to penicillin and other antibiotics. Altogether 75 PRSF isolates were obtained from 56 laboratories. Gemifloxacin had the lowest fluoroquinolone MIC against the 75 PRSF (MIC<sub>90</sub> = 0.25 µg/mL), compared to MIC<sub>90</sub>s of 32 µg/mL for ciprofloxacin, 16 µg/mL for levofloxacin, 8 µg/mL for sparfloxacin and grepafloxacin, and 4 µg/mL for trovafloxacin, gatifloxacin, and moxifloxacin. The number of isolates with mutations in

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both *parC* and *gyrA* increased with increasing ciprofloxacin MICs. In isolates with ciprofloxacin MICs of 16 µg/mL, 12/21 (57%) had mutations in both *parC* and *gyrA* compared to 2/13 (15%) and 1/41 (2.4%) of isolates with a MIC of 8µg/mL and 4 µg/mL for ciprofloxacin, respectively.

In a French study (97), the *in vitro* activity of gemifloxacin was compared with eight other fluoroquinolones against clinical isolates of *S. pneumoniae* from France, PRSF from the Alexander Project, additional French isolates and 28 strains with known mutations in *parC*, *gyrA* and efflux alone or in combination. All clinical strains isolated in France had gemifloxacin MIC of <0.5 µg/mL. For strains with MICs ≥0.5 µg/mL to gemifloxacin, the MIC to trovafloxacin ranged from 2-16 µg/mL, for moxifloxacin the range was 2-4 µg/mL, for sparfloxacin and grepafloxacin the range was 4-32 µg/mL and for all other fluoroquinolones tested, ≥32 µg/mL. No significant difference in fluoroquinolone susceptibility was observed between penicillin-susceptible or -resistant strains. Against 13 well-characterized clinical isolates, gemifloxacin MICs were:

≤0.06 µg/mL for strains carrying mutation(s) in *parE* or *parC* alone; ≤ 0.125 µg/mL for strains carrying mutation(s) in both *parE* and *parC*; and ≤0.5 µg/mL for strains carrying a combination of mutations in *parE*, *parC*, and *gyrA*.

Since the susceptible breakpoint for gemifloxacin will probably be ≤ 0.12 or ≤ 0.25 strains with MICs of ≥ 0.5 µg/mL will not be susceptible to gemifloxacin. The other fluoroquinolones have susceptible breakpoints for *S. pneumoniae* of 1 or 2 µg/mL. Many of these strains with gemifloxacin MICs around 0.5 µg/mL have MICs close to the susceptible breakpoint for most of the newer fluoroquinolones even though gemifloxacin's MIC value is 4- to 8-fold lower. There may be some advantage to using gemifloxacin for strains that have low-level (MICs of 4 to 8 µg/mL) to ciprofloxacin or levofloxacin.

In this same study the impact of efflux mechanisms and various mutations in *parC* and *gyrA*, alone or in combinations was assessed using strains with known mutations. A 2- to 8-fold increase in MIC of all fluoroquinolones was seen in strains with mutations in *parC* at position 79 or 83, alone or in combination; moxifloxacin, sparfloxacin, and levofloxacin showed a small (2-fold) or no increase in MIC. A 4- to 8-fold increase in MIC of gemifloxacin was seen in strains with mutations in *gyrA* at positions 84 and/or 88. A single mutation in *parC* in combination with a single mutation in *gyrA* resulted in an 8- to 32-fold increase in gemifloxacin MIC. When either *parC* or *gyrA* had two mutations in combination with one or more mutations in the other gene the gemifloxacin MIC increased 32- to 512-fold. Gemifloxacin had the lowest MIC value of all the tested fluoroquinolones against these mutants but it also has a susceptible breakpoint that will be 4 to 8-fold lower than that of most of the other fluoroquinolones. When this lower breakpoint is considered the difference among the drugs is not that significant especially for the newer fluoroquinolones. A 4-fold to 8-fold increase in MIC was observed for gemifloxacin and ciprofloxacin, respectively when a strain containing efflux genes was compared with a wild type strain. The other fluoroquinolones tested were not affected by efflux.

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Another study (98) evaluated the antibacterial activity of gemifloxacin and five other fluoroquinolones against wild-type strains of *E. coli*, *S. aureus*, and *P. aeruginosa* and corresponding single-step and multiple-step mutants carrying known resistance mutations in *gyrA* and *parC/grlA* as well as in loci associated with reduced quinolone accumulation (*marR*, *nalB*, *nfxB*, *nfxC*). The MICs of gemifloxacin for fluoroquinolone susceptible and low-level resistant mutants of Gram-negative isolates were comparable to ciprofloxacin and sparfloxacin (within  $\pm 1$  dilution step) and up to 3 dilution steps lower than those of the other fluoroquinolones tested. Gemifloxacin MICs were lower by 1 to 5 dilutions against multiple-step mutants of Gram-negatives. Gemifloxacin MICs were also lower by 1 to 7 dilutions against the parent strain and mutants of *S. aureus*. Gemifloxacin's susceptible breakpoint is 4 to 8 dilutions less than most other fluoroquinolones, however, so this difference probably is not significant clinically for the *S. aureus* and probably means that gemifloxacin will be less active clinically against Gram-negative bacteria. The impact of mutations affecting accumulation was generally low (1 to 3 dilution steps) except for the *P. aeruginosa nfxC* mutant (3-4 steps).

#### Efflux Mechanisms

Efflux mechanisms as a cause of low-level fluoroquinolone resistance have been described in *Streptococcus pneumoniae* and other Gram-positive bacteria. Strains of *S. pneumoniae* exhibiting this form of resistance show reduced susceptibility to hydrophilic fluoroquinolones, such as norfloxacin and ciprofloxacin, but not to hydrophobic fluoroquinolones such as sparfloxacin or moxifloxacin. This resistance can be reversed by the efflux pump inhibitor, reserpine.

MICs to gemifloxacin and five comparator fluoroquinolones were determined in the presence and absence of reserpine against a collection of ofloxacin-resistant (MIC  $\geq 8$   $\mu\text{g/mL}$ ) *S. pneumoniae* (94). Norfloxacin and ciprofloxacin were found to be the most susceptible fluoroquinolones tested, while grepafloxacin and trovafloxacin were the least susceptible to this efflux mechanism. Of the 17 isolates tested, three strains showed a marked level of efflux, such that ciprofloxacin MICs were decreased by 16-fold and norfloxacin MICs decreased 4- to 32-fold by the presence of reserpine. An 8-fold decrease in MIC was reported for gemifloxacin in the presence of reserpine, while little (2-8 fold) or no decrease in MIC to ofloxacin, trovafloxacin, and grepafloxacin were observed in the presence of reserpine. Two isolates showed a 2-fold difference in MIC of norfloxacin, which was, attributed to efflux, however, the other quinolones tested appeared to be unaffected. The rank order of susceptibility to efflux pumps was : ofloxacin = trovafloxacin = grepafloxacin < gemifloxacin < ciprofloxacin = norfloxacin.

In another study (99) gemifloxacin was evaluated for susceptibility to efflux-mediated resistance mechanisms in *S. aureus* and *S. pneumoniae*. These efflux mechanisms, namely the NorA multi-drug transporter in *S. aureus* and the putative PmrA multi-drug transporter in *S. pneumoniae*, contribute to fluoroquinolone resistance in these bacteria. Susceptibility studies demonstrated that bacterial strains over-expressing these transporters had reduced susceptibilities to gemifloxacin. The

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difference in susceptibility between strains over-expressing a multi-drug transporter and their wild-type counterparts was reversed in the presence of NorA inhibitors.

In contrast, Brenwald et al. (100), showed that most strains of *S. pneumoniae* resistant to fluoroquinolones by the PmrA efflux pump remained susceptible to all fluoroquinolones except ciprofloxacin and norfloxacin. Gemifloxacin MICs were  $\leq 0.12 \mu\text{g/mL}$ , except for one strain at  $0.5 \mu\text{g/mL}$ . This mechanism raises gemifloxacin MICs to the susceptible breakpoint.

A further study investigated the rate of selection of resistance to gemifloxacin and ciprofloxacin against *P. aeruginosa*, \_\_\_\_\_ (101). This study showed that the efflux status/multidrug resistance of the bacteria affects the susceptibility of the three species to both gemifloxacin and ciprofloxacin. Resistance to gemifloxacin in *P. aeruginosa* and \_\_\_\_\_ at 3 x MIC occurs at a similar rate to that seen for ciprofloxacin, but at 4 x MIC, resistance to gemifloxacin occurs at a higher rate. Cross-resistance to non-fluoroquinolones in gemifloxacin-resistant strains of \_\_\_\_\_ occurs in 35% of resistant isolates since multi-drug resistance (MDR) efflux systems are the primary mechanism of resistance in these strains.

Transfer of Resistance

Transfer of fluoroquinolone resistance between *S. pneumoniae* and \_\_\_\_\_ as reported in one study (102). One-step fluoroquinolone resistant *ParC* mutants were selected, and QRDRs were transferred from DNA from \_\_\_\_\_ streptococci to *S. pneumoniae* with frequencies of  $10^{-3}$  to  $<10^{-7}$  in correlation with homologies of their QRDR sequences. Reciprocal transfers of mutated *parC* from DNA from *S. pneumoniae* to \_\_\_\_\_ was also observed. Simultaneous transfer of mutated \_\_\_\_\_ to *S. pneumoniae* yielded high-level resistant pneumococci. From this data the authors anticipated the dissemination of QRDRs by efficient inter-species transfer in the clinic. In order for the resistance to be transferred the mutated *parC* gene must be integrated intact into the recipient DNA. When double mutants (*parC/gyrA*) were used as donors high-level resistance was seen only at low frequencies. This suggests that two unlinked genes both have to be integrated.

Another study was performed (103) that contradicts the above study. In this study the ability of *S. pneumoniae* to transfer a gemifloxacin resistant phenotype to gemifloxacin-susceptible strains of *S. pneumoniae* and *S. pyogenes* was investigated. For *S. pneumoniae* to *S. pneumoniae* transfer, a fluoroquinolone-resistant, penicillin-susceptible (PenS) strain was mated with a fluoroquinolone-susceptible, penicillin-resistant (PenR) strain. For *S. pneumoniae* to *S. pyogenes* transfer, a fluoroquinolone-resistant, optochin-sensitive strain of *S. pneumoniae* was mated with a fluoroquinolone-susceptible, optochin-resistant strain of *S. pyogenes*. Transfer of fluoroquinolone resistance by transformation of *S. pneumoniae* by purified DNA was also studied. Inter-species transfer of resistance was not detected by either conjugation or natural transformation. Transfer between *S. pneumoniae* and *S. pyogenes* was also not seen.

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No explanation is given on why the two studies gave different results. It may be that the QRDR genes are not homologous enough between *S. pneumoniae* and *S. pyogenes* for the genetic material that is transferred to be integrated, while these genes are more homologous between *S. pneumoniae* and some                     . This would not, however, explain why transfer between *S. pneumoniae* did not occur.

### Plasmid-mediated Resistance to Fluoroquinolones

Only two reports of plasmid-mediated quinolone-resistance in clinical isolates of                      from Bangladesh (104) and Kashmir (105) exist in the literature. In the first study it was reported that a plasmid found in                      was able to transfer nalidixic acid resistance to                     . It was later shown (106) that the resistance was determined chromosomally and that the presumed transconjugants were probably resistant mutants, because they were selected with nalidixic acid and had no other plasmid-mediated resistances. Ambler et al. (107) confirmed that a plasmid harbored by the Kashmiri strain encoded the mutator phenotype. Ambler and Pinney (108) later showed that the carriage of such mutator plasmids may contribute to the development of quinolone resistance in the clinic. More recently, plasmid-mediated resistance to fluoroquinolones has been described (109) in Gram-negative bacteria. This pMG252 plasmid carries resistance for a number of antibiotics. In this study quinolones were not used to obtain transconjugants, so there was no risk of selection of quinolone-resistant host mutations. This plasmid facilitated selection of higher quinolone resistance but did not enhance mutations generally. Attempts to show decreased quinolone accumulation or drug inactivation by this plasmid have been uninformative. It probably does not provide a quinolone-resistant DNA gyrase or DNA topoisomerase IV, since the chromosomal allele would be dominant and result in quinolone susceptibility. The mechanism of action of this plasmid is a mystery at the present time.

MICs to gemifloxacin, ciprofloxacin, and trovafloxacin were determined against a variety of wild-type and strains harboring mutations, prior to and after the insertion of plasmid pMG252 (110). The introduction of pMG252 into wild-type parent strains resulted in a 4- to 128-fold increase in MIC of all fluoroquinolones tested. Gemifloxacin MICs were similar to those of ciprofloxacin, and the increase in gemifloxacin MIC resulting from the introduction of pMG252 were similar or 2- to 4-fold higher than those of ciprofloxacin. MICs to gemifloxacin were slightly lower than trovafloxacin, but the increase in MICs generally varied 2-fold. The decreased fluoroquinolone activity against strains containing pMG252 is related to the nature of the bacterial host. Strains are likely to be resistant if they already harbor genes conferring resistance to fluoroquinolones. When such mechanisms confer intermediate resistance, the increase caused by pMG252 may be enough to cause clinical resistance.

Enzymatic inactivation of quinolones by bacteria has never been reported.

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SPONTANEOUS EMERGENCE OF RESISTANCE

The prevalence of pre-existing resistant mutants in a bacterial population may be detected by exposing a large inoculum to fluoroquinolone containing media. The frequency at which mutants are isolated is dependent on a number of factors, such as the drug used, the drug concentration used for selection, the species studied, the incubation temperature, the inoculum density, the incubation period, and the incubation conditions. As the concentration of the selecting fluoroquinolone increases above the MIC, the number of resistant mutants identified in the population decreases. If high drug concentrations are used, mutants causing low-level resistance may be unable to grow and selection of low-level resistance will be undetected.

Spontaneous mutation frequencies were determined to gemifloxacin and two comparator fluoroquinolones at 4x, 8x, and 10x the MIC (111) after 48 hours of incubation. A broad spectrum of Gram positive and Gram negative organisms was studied. Against gemifloxacin, trovafloxacin, and ciprofloxacin (4 x MIC), spontaneous mutants of *S. pneumoniae* were selected at a frequency of  $2 \times 10^{-8}$ ,  $3.6 \times 10^{-7}$ , and  $1 \times 10^{-7}$ , respectively. Resistant isolates of *S. pyogenes* were detected at a frequency of  $7 \times 10^{-8}$ ,  $3 \times 10^{-8}$ , and  $2 \times 10^{-8}$  for gemifloxacin, trovafloxacin, and ciprofloxacin, respectively at 4 x MIC. For *K. pneumoniae* and \_\_\_\_\_ spontaneous mutants were detected at 4x, 8x, and 10x MIC against all three drugs at a rate of  $<2.2 \times 10^{-8}$ . *S. aureus*, \_\_\_\_\_ mutants resistant to ciprofloxacin were selected at  $<6.7 \times 10^{-7}$  using 4 x MIC drug concentration. Overall, the frequency of spontaneous resistance to gemifloxacin ranged from  $<1 \times 10^{-8}$  to  $8 \times 10^{-7}$ . Spontaneous mutants were selected more frequently with ciprofloxacin than with gemifloxacin or trovafloxacin.

In another study (112) the frequency of spontaneous resistance to gemifloxacin and two other fluoroquinolones was determined at 2x, 4x, and 8x MIC using 49 clinical isolates of *S. pneumoniae* and 24 and 48 hours of incubation. A mean rate of spontaneous mutation for the 49 isolates was determined at 4 x MIC: gemifloxacin  $6.78 \times 10^{-7}$ ; levofloxacin  $9.23 \times 10^{-8}$ ; and ciprofloxacin  $2.73 \times 10^{-8}$ . The differences in rates between gemifloxacin and levofloxacin were significant ( $p < 0.01$ ), but not between gemifloxacin and ciprofloxacin. A four-fold increase in MIC during serial transfer occurred most rapidly with gemifloxacin and least rapidly with levofloxacin. These frequencies vary by  $1 \log_{10}$  for gemifloxacin and  $2 \log_{10}$  for ciprofloxacin compared to the previous study (111).

In yet another study (113) the rate of spontaneous resistance to gemifloxacin, trovafloxacin, and ciprofloxacin was determined against nine species at 4x, 8x, and 10x MIC after 24 hours incubation. Of the species tested, mutants resistant to all three drugs were selected with *K. pneumoniae*. Gemifloxacin selected mutants to the least number of species tested. Resistant mutants were selected with gemifloxacin at 4 x MIC for only two of the nine species tested (*K. pneumoniae* at a frequency of  $4.6 \times 10^{-7}$  and *S. pneumoniae* at a frequency of  $1.7 \times 10^{-8}$ ). Trovafloxacin and ciprofloxacin selected resistant mutants at 4 x MIC for 6/9 and 4/9 species, respectively. The frequencies of resistant mutants for trovafloxacin for *K. pneumoniae* ( $4 \times 10^{-7}$ ) and *S. pneumoniae* ( $9 \times 10^{-8}$ ) were comparable to those for gemifloxacin. Ciprofloxacin's frequency for

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*K. pneumoniae* ( $2.9 \times 10^{-7}$ ) was comparable to that of gemifloxacin's frequency for this species. Ciprofloxacin's frequency for *S. pneumoniae* ( $<2.9 \times 10^{-9}$ ) was about 1 log<sub>10</sub> lower than that of gemifloxacin.

The frequency of spontaneous mutation, cross-resistance of mutants to other antibiotics, and development of stepwise resistance to gemifloxacin were examined against strains of eight species (114). The frequency of spontaneous mutations to gemifloxacin was similar to that of the other fluoroquinolones tested. Repeated subculture in the presence of increasing concentrations of drug raised the MIC of gemifloxacin from 4- to 128-fold. The MICs of these mutants were still below or at the susceptible breakpoint with the exception of \_\_\_\_\_ (gemifloxacin's MIC went from 1 µg/mL to 128 µg/mL). Resistance seemed to develop faster for this species against gemifloxacin than against sparfloxacin or ciprofloxacin. All resistant isolates selected with gemifloxacin or ciprofloxacin were cross-resistant with one another.

A further study (115) investigated the rate of development of *in vitro* resistance to gemifloxacin, ciprofloxacin, and trovafloxacin in \_\_\_\_\_ and *S. aureus* strains. Using inocula of  $10^9 - 10^{10}$  cfu of \_\_\_\_\_, 8 x MIC of gemifloxacin and ciprofloxacin, or 4 x MIC of trovafloxacin were needed to prevent emergence of resistant mutants. The maximum increase in MIC was 16-fold for gemifloxacin, 8-fold for ciprofloxacin, and 4-fold for trovafloxacin compared to wild-type. With the same inoculum of *S. aureus*, 2 x, 4 x, and 16 x MIC of gemifloxacin, ciprofloxacin, and trovafloxacin, respectively, were needed to prevent emergence of resistant mutants and the maximum increase in MIC was 4-fold for gemifloxacin, 16-fold for ciprofloxacin, and 8-fold for trovafloxacin.

In a similar study (116), spontaneous resistant rates were determined for gemifloxacin, trovafloxacin, and ciprofloxacin to first step mutations in *S. pneumoniae*. The concentrations used were 1x, 2x, 4x, 8x, and 16x MIC of each drug. The isolates were incubated for 48-72 hours. Mutation rates at 4x MIC ranged from  $<6.72 \times 10^{-9}$  to  $<1.0 \times 10^{-10}$  for gemifloxacin,  $4.6 \times 10^{-7}$  to  $<2.5 \times 10^{-10}$  for ciprofloxacin, and  $3.0 \times 10^{-9}$  to  $<2.0 \times 10^{-10}$  for trovafloxacin. At  $\geq 4$  x MIC gemifloxacin in all cases but one had the lowest single-step mutation frequency rates compared to the other two drugs. At the MIC and 2 x MIC gemifloxacin had the lowest single-step mutation frequency rates in almost all cases.

It appears that for most species the spontaneous resistant rate for gemifloxacin is comparable to that for ciprofloxacin and trovafloxacin. Some experiments show that gemifloxacin has a lower rate against *S. pneumoniae* when compared to ciprofloxacin. In some experiments trovafloxacin (or levofloxacin) had a lower frequency than gemifloxacin and in other studies gemifloxacin had a slightly lower frequency.

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**DEVELOPMENT OF RESISTANCE BY SERIAL PASSAGE**

Ten isolates were passaged in the presence of sub-inhibitory concentrations (0.5 x MIC) of gemifloxacin, trovafloxacin, and ciprofloxacin. Baseline MICs and subsequent passages MICs were determined using a macrodilution method. Following 5, 10, and 15 daily passages, MICs were determined and the difference from baseline MIC values was noted. A increase of >4-fold from the baseline was considered significant. After 5 passages, no significant change in the gemifloxacin MIC was seen against 7/10 of the isolates tested, compared to 5/10 for both trovafloxacin and ciprofloxacin. After 10 passages no significant change in gemifloxacin MIC was observed against 6/10 isolates, compared to 3/10 for both trovafloxacin and ciprofloxacin. After 15 passages, the MIC of gemifloxacin and trovafloxacin increased  $\geq$ 8-fold against 7/10 isolates, and the MIC of ciprofloxacin increased  $\geq$ 8-fold against 8/10 isolates. These results indicate that repeated exposure of isolates to sub-inhibitory concentrations of fluoroquinolones leads to reduced susceptibilities. In this study, resistance to trovafloxacin and ciprofloxacin developed more rapidly than with gemifloxacin. The results are summarized in TABLE 104.

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TABLE 104  
Number of Dilution increase in MIC following passages in sub-inhibitory concentrations

Isolate	Passage (days)	Fold Increase in MIC (MIC, µg/mL)		
		Gemifloxacin	Trovafloxacin	Ciprofloxacin
<i>S. aureus</i> ATCC 29213	5	0 (0.06)	2 (0.12)	4(2)
	10	2 (0.12)	4 (0.25)	16 (8)
	15	2 (0.12)	2 (0.12)	64 (32)
<i>S. pneumoniae</i> ATCC 49619	5	4 (0.03)	16 (2)	2 (1)
	10	32 (0.25)	256 (32)	16 (8)
	15	32 (0.25)	512 (64)	128 (64) <sup>b</sup>
<i>H. influenzae</i> ATCC 49247	5	8 (0.03)	8 (0.06)	64 (0.5)
	10	32 (0.12)	16 (0.12)	256 (2)
	15	128 (0.5) <sup>b</sup>	16 (0.12)	1024 (8)
<i>K. pneumoniae</i> E70	5	8 (4)	64 (32)	16 (4)
	10	16 (8)	64 (32)	32 (8)
	15	>512 (>256)	64 (32)	32 (8)

a. based on 13 passages, b. Based on 14 passages

These data show that *P. aeruginosa* and *K. pneumoniae* develop resistance more readily than most other species. This has been seen with almost all of the fluoroquinolones. It appears that *H. influenzae* may also develop resistance faster than some other species against gemifloxacin and ciprofloxacin. This is not as big a problem with trovafloxacin.

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Another study investigated the development of step-wise resistance by repeated exposure to increasing concentrations of gemifloxacin, sparfloxacin, and ciprofloxacin (114). Test organisms (*S. aureus*, *S. pneumoniae*, \_\_\_\_\_) were grown in Mueller-Hinton broth and inoculated into fresh drug-containing broth at 2-fold incremental concentrations. From the highest concentration showing visible growth, approximately  $1.5 \times 10^5$  cfu were re-exposed to 2-fold incremental concentrations until the concentration above which further increase did not occur was reached. This experiment showed that exposure of bacteria to increasing concentrations of gemifloxacin, sparfloxacin, or ciprofloxacin resulted in the selection of organisms with higher MICs. Prior to exposure to drug, the MIC of gemifloxacin for both *S. aureus* and *Streptococcus pneumoniae* was 0.016  $\mu\text{g}/\text{mL}$ . After 7 transfers, the MICs of gemifloxacin were 0.13 and 0.06  $\mu\text{g}/\text{mL}$ , respectively. There was an eight-fold increase in the MIC of gemifloxacin for *S. aureus* and a four-fold increase in the MIC for *S. pneumoniae*. The MICs of sparfloxacin increased from 0.13 to 4  $\mu\text{g}/\text{mL}$  (32-fold increase) for *S. aureus* and from 0.25 to 1  $\mu\text{g}/\text{mL}$  (4-fold increase) for *S. pneumoniae* after 7 transfers. After 7 transfers the ciprofloxacin MIC for *S. aureus* increased from 0.25  $\mu\text{g}/\text{mL}$  to 1  $\mu\text{g}/\text{mL}$  (4-fold increase) and the MIC for *S. pneumoniae* went from 0.5 to 1  $\mu\text{g}/\text{mL}$  (2-fold increase). It appears that the amount of increase was about equal, especially for *S. pneumoniae*. Although gemifloxacin MICs remained low, most are just below or at the susceptible breakpoint. Most of the MICs for the two comparator drugs were at or just above the susceptible breakpoints after 7 transfers. \_\_\_\_\_ a 16-fold increase (from 0.016 to 0.25  $\mu\text{g}/\text{mL}$ ) in the MIC of gemifloxacin. The \_\_\_\_\_ Ciprofloxacin MICs increased from 0.008  $\mu\text{g}/\text{mL}$  to 1  $\mu\text{g}/\text{mL}$ . The increases for both gemifloxacin and ciprofloxacin gave MICs at the susceptible breakpoint for each drug after 7 transfers.

\_\_\_\_\_ Gemifloxacin induced resistance more rapidly than sparfloxacin or ciprofloxacin.

A similar study (118) investigated the ability of gemifloxacin, ciprofloxacin, and trovafloxacin to select for pneumococcal mutants with elevated MICs by serial passage. Four ciprofloxacin susceptible strains (ciprofloxacin MIC range 0.5-1  $\mu\text{g}/\text{mL}$ ) and eight strains with elevated ciprofloxacin MICs (MIC range 8-32  $\mu\text{g}/\text{mL}$ ) were tested. Serial passage resulted in the selection of resistant mutant strains for all fluoroquinolones tested. Subculturing in ciprofloxacin led to selection of mutants with raised ciprofloxacin MICs as follows: among the four strains with initial ciprofloxacin MICs of 0.5-1.0  $\mu\text{g}/\text{mL}$  all strains became resistant (MICs  $>4$   $\mu\text{g}/\text{mL}$ ) after 4-16 passages and among the eight strains with initial ciprofloxacin MICs of 8-32  $\mu\text{g}/\text{mL}$  all strains had ciprofloxacin MICs increase  $\geq 4$ -fold to 64-128  $\mu\text{g}/\text{mL}$  after 4-13 passages. Subculturing in trovafloxacin led to selection of mutants with raised trovafloxacin MICs as follows: among the ten strains with initial trovafloxacin MICs of 0.125-2  $\mu\text{g}/\text{mL}$  eight strains became fully resistant to trovafloxacin (MICs  $\geq 4$   $\mu\text{g}/\text{mL}$ ) after 4-33 passages and among the two strains initially resistant to trovafloxacin (MICs of 4  $\mu\text{g}/\text{mL}$ ) both strains had trovafloxacin MICs increase

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4-fold to 16 µg/mL after 14-40 passages. Subculturing in gemifloxacin led to selection of mutants resistant to gemifloxacin in all 12 strains with gemifloxacin MICs rising from 0.015-0.25 µg/mL to ≥2 µg/mL after 5-29 subcultures. This experiment assumed a susceptible breakpoint of 0.5 µg/mL for gemifloxacin. This is too high a value. The susceptible breakpoint for *S. pneumoniae* should be 0.12 µg/mL which would make strains with MICs of 0.5 µg/mL resistant and not those at 2 µg/mL. It appears that gemifloxacin and trovafloxacin act about the same in this experiment. Since most strains are already close to the ciprofloxacin susceptible breakpoints it only takes a few (<10) passages to increase the MIC to values ≥8 µg/mL.

A further study (112) investigated the development of resistance to fluoroquinolones through serial passage against 49 clinical isolates of *S. pneumoniae*. The gemifloxacin MICs increased 4-fold in 55% of strains tested following a single passage and increased 4-fold after two passages against the remaining 45%. By contrast, levofloxacin MICs increased 4-fold in 35% of strains following two passages and six passages were needed to increase MICs of all isolates 4-fold. Ciprofloxacin MICs increased 4-fold in over 55% of strains after two passages and in all strains after seven passages. In this experiment higher MICs developed more rapidly when gemifloxacin was the test drug than when levofloxacin or ciprofloxacin were used. Gemifloxacin MICs were lower (range 0.008-0.25 µg/mL) compared with levofloxacin and ciprofloxacin (0.5-2 µg/mL), however, the higher MIC values were at approximately the susceptible breakpoint for each drug.

Development of resistance to gemifloxacin, trovafloxacin, erythromycin, and doxycycline was investigated in \_\_\_\_\_ by serial passage (119). The two strains that were studied developed resistance to all four drugs tested during eleven passages in drug-containing broth. Resistance to gemifloxacin, trovafloxacin, or erythromycin was apparent after seven to eight passages, while resistance to doxycycline was detected within two passages. The degree and rate of resistance development to gemifloxacin, trovafloxacin, and erythromycin were very similar.

These studies demonstrate that gemifloxacin acts much like other fluoroquinolones in respect to development of resistance by serial passage. Most experiments show that the rate of resistance development is similar to most other fluoroquinolones. In one experiment gemifloxacin seemed to develop resistance at a somewhat lower rate (based on the -fold increase in MICs) but in another experiment using *S. pneumoniae* the rate of resistance development was faster for gemifloxacin than for levofloxacin or ciprofloxacin. The rate of resistance development against \_\_\_\_\_ seems to be similar to ciprofloxacin.

As with most other fluoroquinolones *K. pneumoniae* and \_\_\_\_\_ seem to develop resistance more readily than most other species. It appears from data in one study that *H. influenzae* may develop resistance more easily to gemifloxacin than other fluoroquinolones.

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DEVELOPMENT OF RESISTANCE DURING CLINICAL TRIALS

Increases in MIC and decreases in disk zone size diameter for gemifloxacin and comparators were monitored during the phase III clinical trials. All of the isolates from patients showing an apparent increase in MIC of 4-fold or more between initial screening and end of therapy or post-therapy visits, or a decrease in disk zone diameter of 6 mm or more for gemifloxacin or comparator were evaluated. A total of 8,360 patients were included in the clinical trials of which 219 (3%) from either group (gemifloxacin or comparator) had isolates that met the above criteria. Antimicrobial susceptibility retesting was performed on isolates that met the above criteria at the four central laboratories that performed the original susceptibility testing. Isolates from 55 patients had changes for the comparator drug only. Isolates or complete retest data were not available for 74 patients and these isolates were not further characterized. Isolates from 63 of the remaining 90 patients were not further studied since the apparent changes in MIC or disk diameters were not observed between screening and end of therapy or post-therapy isolates. Isolates from 20 of the remaining 27 patients demonstrated an increase in MIC of < 4-fold or a decrease in disk diameter < 6 mm, respectively, to gemifloxacin upon retest. Further testing (pulse-field gel electrophoresis--PFGE) showed that 6 out of the 7 remaining patients had isolates that were either different species of the same genus or different strains of the same species. Only one patient had screening and end of therapy or post-therapy isolates that were identified as the same. Both isolates from this patient had mutations in the quinolone resistance determining region. Additional mutations were found in the post-therapy isolate. There were 101 patients that had the required changes in gemifloxacin values between the required visits. This high a number of patients with isolates with increased MIC values is unusual for fluoroquinolones. Usually less than 20 such isolates are seen. This submission, however, has about 2.5 to 3 times the number of patients that are usually seen in fluoroquinolone NDA submissions. From the entire patient population, 1% of the isolates with apparent changes in MIC or disk susceptibility were from patients treated with gemifloxacin, compared to 1% treated with a comparator. When those studies are considered in which the comparator drug was a quinolone, 2% of the isolates were from patients treated with gemifloxacin and 1% from patients treated with comparator drugs. These percentages are close to those usually observed. TABLE 105 summarizes what happened to the 219 patients identified with MIC or zone diameter changes.

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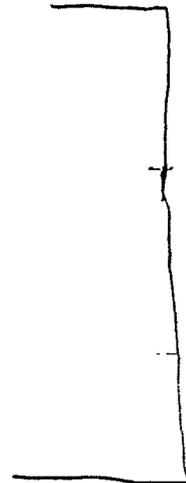
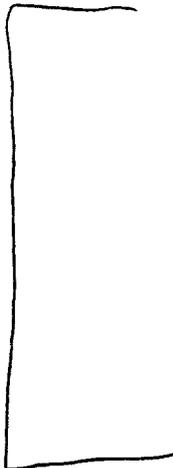
The \_\_\_\_\_ isolate had an initial MIC of 0.008 µg/mL and a MIC of 0.03 µg/mL at post-therapy. The initial isolate had a L41F mutation in gyrA and a Y421F mutation in GyrB. The post-therapy isolate had these mutations plus a R43H mutation in ParC and a D87G mutation in GyrA.

TABLE 107 presents organisms with gemifloxacin MICs or zone diameters that met the criteria and differences were observed between screening (V1) and end-of-therapy (V3) or post-therapy (V4). The shaded isolates were the ones with retest data. The retest values are in parentheses after the original values.

**TABLE 107  
Organisms with Gemifloxacin MICs or Zone Diameters that Indicate Resistance  
Development during Treatment**

Organism	Baseline MIC	Post-therapy MIC	Baseline Zone Diameter	Post-therapy Zone Diameter
<i>Haemophilus influenzae</i> (n= 4)			33	24
<i>Haemophilus influenzae</i>	0.002	0.008		
<i>Haemophilus influenzae</i>	0.002 (0.002)	0.015 (0.002)	46 (32)	38 (32)
<i>Haemophilus influenzae</i>			41 (40)	33 (41)
<i>Haemophilus parainfluenzae</i> (n=10)		39 (33)	32 (34)	
<i>Haemophilus parainfluenzae</i>			31 (31)	28 (29)
<i>Haemophilus parainfluenzae</i>	0.004	0.12	35	25
<i>Haemophilus parainfluenzae</i>			34	27
<i>Haemophilus parainfluenzae</i>	0.03 (0.03)	0.12 (0.06)		
<i>Haemophilus parainfluenzae</i>			35 (27)	28 (26)
<i>Haemophilus parainfluenzae</i>			28 (28)	22 (22)
<i>Haemophilus parainfluenzae</i>	0.015 (0.015)	0.06 (0.03)		
<i>Haemophilus parainfluenzae</i>			33	18
<i>Haemophilus parainfluenzae</i>	≤0.001	0.008		

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TABLE 107 (continued)  
Organisms with Gemifloxacin MICs or Zone Diameters that Indicate Resistance  
Development during Treatment

Organism	Baseline MIC	Post-therapy MIC	Baseline Zone Diameter	Post-therapy Zone Diameter
<i>Klebsiella pneumoniae</i> (n=4)			47	26
<i>Klebsiella pneumoniae</i>			28	21
<i>Klebsiella pneumoniae</i>			27	21
<i>Klebsiella pneumoniae</i>	0.03	0.25		
<i>Streptococcus pneumoniae</i> (n = 5)		37	29	
<del><i>Streptococcus pneumoniae</i></del>	<del>0.015 (0.015)</del>	<del>0.08 (0.015)</del>		
<i>Streptococcus pneumoniae</i>	0.002	0.015		
<i>Streptococcus pneumoniae</i>			39	28
<del><i>Streptococcus pneumoniae</i></del>			<del>33 (30)</del>	<del>26 (28)</del>

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